REMARKS

The present application is directed to a method for detecting the presence of a target nucleic acid sequence in a sample by amplifying the target to produce an amplification reaction product that includes a purine rich region, contacting the sample during the amplification with a peptide nucleic acid able to bind to at least a portion of the target sequence and detecting the presence of triplex DNA structures. The application is also directed to a kit containing a bis-peptide nucleic acid (PNA) sequence designed to form a triplex with a target sequence and a set of amplification primers that can amplify a sequence including the target sequence.

Upon entry of the amendment, Claims 1-2, 5-6, 8-12, 18-19 and 22-26 will be pending. Claims 3-4, 7, 13-17, 20 and 21 have been cancelled without prejudice. Claims 6, 18-19 and 25 are currently amended. Support for the amendments can be found in cancelled Claims 3 and 20.

Rejection Under 35 U.S.C. 103(a)

In the Office Action mailed December 27, 2006, the Examiner rejected Claims 6 and 22-24 under 35 U.S.C. § 103(a) as obvious over Vary *et al.* (U.S. Patent No. 5,800,984; hereinafter "Vary") in view of Egholm *et al.* (WO 96/02558 A1; hereinafter "Egholm"). Applicants respectfully submit that the amendments to the claims overcome the rejection.

Claim 6 has been amended to clarify that the sample is contacted **during the amplification** with the peptide nucleic acid. Claims 22-24 depend from amended Claim 6. The Examiner was persuaded to withdraw the rejection of Claims 1,5-6, 8-9, 12 and 22-24 when the same amendment was made to Claims 1 and 18 in the Amendment and Response to Office Action filed October 4, 2007. Therefore, now that Claims 6 and 22-24 contain the **same** limitation, applicants respectfully request the withdrawal of the rejection.

Vary teaches a method for detecting a nucleic acid target sequence by formation of a triplex structure. However, Vary fails to teach or suggest the use of a **PNA** probe to form this structure.

Egholm teaches novel PNAs such as bis-PNA. However, Egholm fails to teach formation of a triplex structure during the amplification as set forth in amended Claim 6. In fact, applicants respectfully submit that Egholm **teaches away** from using PNA probes during an **amplification** reaction because Egholm teaches that PNA binds nucleic acid molecules with a high **affinity**, which should **impede** amplification.

Egholm states that PNA binds both DNA and RNA with great affinity. (See page 3, lines 15-18.) Egholm also explains that PNAs have been used to detect point mutations in PCR-based assays, using a technique known as "PCR clamping". (See page 5, lines 9-21). In PCR clamping, PNA is used to detect point mutations by preferentially binding to target DNA. In the absence of a point mutation in the target sequence, PCR amplification is **prevented** by the binding of the PNA to the target DNA. One skilled in the art would deduce from Egholm that a triplex containing PNA would be so highly **stable** that it would effectively **clamp** amplification of the product. Rather than being motivated to combine the cited references, one skilled in the art would have **avoided** use of a PNA probe during amplification of a target sequence due to the high **affinity** of PNA for DNA and the anticipated **clamping** of amplification as noted by Egholm.

Accordingly, applicants respectfully request withdrawal of the Examiner's rejection of Claims 6 and 22-24 under 35 U.S.C. §103(a) as obvious over Vary in view of Egholm.

The Examiner rejected Claims 1, 2, 5, 8-9 and 12 under 35 U.S.C. § 103(a) as obvious over Vary in view of Egholm and newly cited Livak *et al.* (U.S. Patent No. 5,723,591; hereinafter "Livak"). Applicants respectfully traverse.

Claim 1 is directed to a method for detecting the presence of a target nucleic acid sequence in a sample by amplifying the target nucleic acid and contacting the sample during amplification with a PNA that binds to at least a portion of the target, and detecting the presence of triplex structures. Claims 2, 5, 8-9 and 12 depend from Claim 1.

The teachings of Vary and Egholm are discussed above.

Livak teaches use of an oligonucleotide probe that includes a fluorescent reporter molecule and a quencher molecule. The quencher molecule is capable of quenching the fluorescence of the reporter for use in monitoring nucleic acid amplification.

As explained above, one skilled in the art would have expected that the stability of a triplex formed between the amplified target sequence and the PNA would be so **high** that it would effectively **clamp** further amplification of the product. Rather than being motivated to combine the cited references, applicants respectfully submit that one skilled in the art would have **avoided** the use of a PNA probe during amplification because, despite the known advantages of real-time PCR, in which an oligonucleotide probe is present during amplification, it would be technically **disadvantageous** to use a **PNA** probe during amplification of a target sequence due to the high **affinity** of PNA for DNA, as noted by Egholm.

Evidence published in the scientific literature **after** the present method was developed has shown that nucleic acid amplification can be achieved in the presence of a complementary PNA. However, those skilled in the art found this result **surprising** in view of the known properties of PNA. For example, Wolffs *et al.* (2001; *BioTechniques* 31:766-771; previously submitted) used PNA conjugated to thiazole orange to form a "light-up" probe for use in real-time PCR and concluded that the PNA light-up probes "do **not** inhibit DNA amplification, as might have been **expected** from PCR clamping studies" (page 270, final paragraph) (emphasis added).

Accordingly, applicants respectfully request withdrawal of the Examiner's rejection of Claims 1, 2, 5, 8-9 and 12 under 35 U.S.C. §103(a) as obvious over Vary in view of Egholm and Livak.

The Examiner rejected Claims 10-11 and 18-19 under 35 U.S.C. § 103(a) as obvious over Vary in view of Egholm and Livak, as applied to Claims 1, 2, 5, 8-9 and 12 above, and further in view of Graham *et al.* (WO 97/05280; hereinafter "Graham"). Applicants respectfully traverse.

Claims 10-11 depend from Claim 1. Claim 10 specifies that the PNA is immobilized on a waveguide of a detection device. Claim 11 specifies that the detection device is a surface plasmon resonance detector.

Independent Claim 18 is directed to a method for detecting the presence of a target nucleic acid sequence in a sample by amplifying the target nucleic acid and contacting the sample during amplification with a waveguide of an evanescent waveguide detector on which is immobilized a PNA that binds to at least a portion of the target, and detecting the presence of triplex structures. Claim 19 depends from Claim 18 and specifies that the waveguide detector is a surface plasmon resonance detector.

The teachings of Vary, Egholm and Livak are discussed above.

Graham describes a method for detecting a target nucleic acid in a sample by binding a label to a surface enhanced (resonance) Raman scattering surface, attaching a PNA probe to the surface, wherein the PNA probe is designed to react with the target of interest, and obtaining a spectrum, referred to as an SER(R)S spectrum. In this context, Graham teaches that the PNA probe would "bind to DNA to form hybrids which are more stable than the corresponding DNA-DNA hybrids" (page 39, second paragraph). Graham also teaches that PNA "would be expected to be less acidic than the corresponding wild-type DNA or RNA sequence and would therefore show an increased affinity for a SER(R)S-active surface" (page 40, lines 1-4).

As explained above with respect to Claim 1, applicants respectfully submit that, far from having a reasonable expectation of success, it would be more reasonable for one of ordinary skill in the art to deduce from the cited references that the **stability** of a triplex formed between the amplified target sequence and the PNA would be so **high** that it would effectively **clamp** further amplification of the product. Rather than being motivated to combine the cited references, applicants respectfully submit that one of ordinary skill in the art would consider it technically **disadvantageous** to use a PNA probe for a target sequence during amplification of that target sequence due to the high **affinity** of PNA for DNA, as noted by Egholm.

Clearly, one of ordinary skill in the art at the time the invention was made would **not** have expected amplification to work in the presence of a PNA – the fact that the PNA is in solution or is immobilized would not change this expectation.

Graham adds **nothing** to change the expectation of one of ordinary skill in the art that PNA would **inhibit** amplification. Graham confirms the concept that PNA has an increased affinity for DNA or RNA sequences and that PNA-DNA hybrids are highly stable. Therefore, Graham also **teaches away** from the use of PNA **during amplification**.

Accordingly, applicants respectfully request withdrawal of the rejection of Claims 10-11 and 18-19 under 35 U.S.C. § 103(a) as obvious over Vary, Egholm, and Livak and further in view of Graham.

The Examiner rejected Claims 25-26 under 35 U.S.C. § 103(a) as obvious over Graham in view of Egholm. Applicants respectfully submit that the amendments to the claims overcome the rejection.

Independent Claim 25 is directed to a kit for detecting the presence of a target nucleic acid in a sample, wherein the kit contains an immobilized bis-PNA on a waveguide of an evanescent wave detector apparatus and also contains a set of amplification primers that amplify target in the presence of a bis-PNA sequence. Claim 25 has been amended to specify that the bis-PNA can form a PNA_2DNA triplex structure with the target nucleotide sequence **during amplification** of the target nucleotide sequence. Claim 26 depends from Claim 25 and specifies that the detector is a surface plasmon resonance detector.

The teachings of Graham and Egholm are discussed above.

Applicants respectfully submit that neither Graham nor Egholm teach, suggest or even imply a kit containing **bis-PNA** immobilized on a waveguide of an evanescent wave detector wherein the bis-PNA can form a PNA_2DNA triplex structure with the target nucleotide sequence **during amplification**.

As discussed above, one skilled in the art would conclude from the teachings of the cited references that the **stability** of a triplex formed between an amplified target sequence and a PNA molecule would be so **high** that it would effectively **clamp** further amplification of

the product and would consider it technically **disadvantageous** to use a PNA probe for a target sequence **during** amplification. In view of those teachings, one skilled in the art would have no interest in assembling a kit containing the components set forth in Claim 25. The cited references fail to describe a kit containing a **bis-PNA** that can form a triplex structure with the target nucleotide sequence **during amplification**. Therefore, it would not be obvious to assemble a kit containing this bis-PNA and the specific amplification primers recited. One skilled in the art would avoid assembly of the specific bis-PNA and primers in a kit because it was taught that PNA would inhibit nucleic acid amplification.

Accordingly, applicants respectfully request withdrawal of the rejection of Claims 25 and 26 under 35 U.S.C. §103(a) as obvious over Graham in view of Egholm.

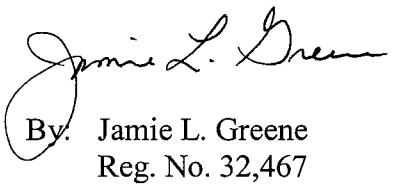
CONCLUSION

In light of the amendments and the above remarks, applicants are of the opinion that the Office Action has been completely responded to and that the application is now in condition for allowance. Such action is respectfully requested.

If the Examiner believes any informalities remain in the application that may be corrected by Examiner's Amendment, or there are any other issues that can be resolved by telephone interview, a telephone call to the undersigned attorney at (404) 815-6500 is respectfully requested.

No additional fees are believed due, however, the Commissioner is hereby authorized to charge any deficiencies that may be required, or credit any overpayment, to Deposit Account Number 11-0855.

Respectfully submitted,



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